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Research Article

Admixture between lineages of the tramp ant *Technomyrmex albipes* revealed by mitochondrial and ultra-conserved element sequencing

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Abstract

Hybridisation between divergent populations can lead to various outcomes, including potentially increased invasiveness. In this study, we investigated the genetic background of a recent outbreak of a tramp ant species in southern Taiwan. Combining morphological data and a global *Technomyrmex* phylogeny, based on 1,796 newly-generated and publicly-available *cytochrome c oxidase I* (COI) barcodes, we identified the species responsible for the outbreak as *Technomyrmex albipes*. Further haplotype network analysis, based on 399 *T. albipes* samples, revealed two major geographic groups corresponding to the Indian Ocean Region and Southeast Asia. Population structure inferred from ultra-conserved element sequence data revealed that two genetically similar lineages are geographically separated by a third lineage in western Taiwan, indicating a potential human-mediated dispersal event. We found that one of the contact zones between these lineages coincides with the region where outbreaks were reported. Several individuals in this area exhibited admixed genotypes and this potential hybridisation was further supported by simulations. Based on these findings, we proposed that the outbreaks are potentially attributable to recent admixture between lineages in southern Taiwan.

Key words: *Cytochrome c oxidase I* barcode, population genomics, simulation, Taiwan

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Introduction

Human-aided dispersal of tramp species has been a primary concern during the era of globalisation (Meyerson and Mooney 2007; Hulme 2009). Arrival of these exotic species can have profound ecological effects on native environments through interacting with native species. These interactions may include competition, predation and hybridisation (Mooney and Cleland 2001). Competitive displacement or predation often leads to declines in native populations (Holway et al. 2002; Colvin et al. 2005; Bøhn et al. 2008), whereas hybridisation can result in more diverse outcomes (Barton 2001). Hybridisation following the introduction of exotic species may occur

in various configurations, depending on the taxonomic relationship (interspecific or intraspecific) and geographic origin (native × exotic or exotic × exotic) of the two populations (Mooney and Cleland 2001; Lee 2002; Schierenbeck and Ellstrand 2009). Hybrid individuals may suffer from outbreeding depression due to the loss of local adaptations (Levin et al. 1996; Rhymer and Simberloff 1996) or, conversely, they may gain increased adaptability, enabling them to demographically overwhelm the individuals with pure native genetic content (Ellstrand and Schierenbeck 2000; Lee 2002; Verhoeven et al. 2010). Therefore, although hybridisation does not necessarily promote invasiveness (Wolfe et al. 2007; Culley and Hardiman 2009; Szűcs et al. 2021), several studies have shown that admixed genotypes may offer ecological advantages over their parental genotypes (Whitney and Gabler 2008; Schierenbeck and Ellstrand 2009). One example is the Africanised honeybee in the Americas, a hybrid between the introduced African subspecies and local European-derived subspecies. These hybrids exhibit increased aggression, higher colony productivity and greater tolerance to parasites and, therefore, have displaced European honeybee populations across much of their introduced range (Winston 1992; Martin and Medina 2004; Schneider et al. 2004; Pinto et al. 2005).

In ants, many species have been documented to successfully establish colonies outside of their native ranges (McGlynn 1999; Lowe et al. 2000; Heinze et al. 2006; Tseng et al. 2023), but most of the studies focused on species that cause serious damage, such as the red imported fire ant (*Solenopsis invicta*) and the Argentine ant (*Linepithema humile*) (Suarez et al. 2010). *Technomyrmex* ants are one of the overlooked pest groups, although these ants have been frequently reported to invade greenhouses or botanical gardens (Bolton 2007; Wetterer 2013; Pech and Bezděk 2016). Unlike most ant species, where typical winged queens are responsible for reproduction, some *Technomyrmex* species, particularly those from the *albipes* group, employ a dual winged-wingless reproductive system (Tsuji et al. 1991; Yamauchi et al. 1991; Ogata et al. 1996; Bolton 2007). This unique reproductive system is characterised by a process whereby multiple queen-worker intermorphic females (intercastes) take over the colony after the queen's early death and reproduce through inbreeding with wingless males (Yamauchi et al. 1991). Despite inbreeding and over-representation of reproductive castes within the nest, this system may act as a pre-adaptation for overcoming genetic bottlenecks and low propagule pressure that are often encountered during invasion (Eyer et al. 2018; Eyer and Vargo 2021; Lin et al. 2025). Many *Technomyrmex* species are, therefore, capable of colonising new environments with a random fraction of the mother colony.

Indeed, some *Technomyrmex* species from the *albipes* group have become worldwide tramp species (Bolton 2007; Wetterer 2013; Janicki et al. 2016). Although they do not typically pose direct threats to humans or native fauna like some other invasive ants (Jemal and Hugh-Jones 1993; Wetterer and Porter 2003; Lee and Yang 2022), their invasive traits are still evident. Several species within this group have already been recorded from and are established in various regions outside their native ranges (Wetterer 2013; Pech and Bezděk 2016; Sharaf et al. 2018; Väänänen et al. 2018; Barros et al. 2022; Pawluk et al. 2022), with some attaining severe pest status (Warner 2003; Putri et al. 2021; Terayama et al. 2021; Putri and Cronin 2023; Hsu et al. 2024). Owing to their similar external morphologies, however, numerous taxonomic challenges have emerged, leading to frequent misidentifications and confusion between local and tramp species (Bolton 2007). For example, *T. albipes* has often been confused with other tramp species, such as *T. difficilis* and

T. vitiensis, as well as locally distributed species, such as *T. pallipes* from the Afrotropical Region and *T. brunneus* from the Oriental Region (Bolton 2007).

Effective monitoring and management of invasive species rely heavily on accurate species identification; misidentification could potentially lead to biased abundance records at ports of entry and even control failures (Venette et al. 2021). This challenge is particularly pronounced for *Technomyrmex* species due to the rarity of phylogenetically informative diagnostic characters (Bolton 2007). Several studies have highlighted this challenge. For example, Wetterer (2013) noted that even experienced myrmecologists can misidentify tramp species from the *albipes* group, based solely on morphology. Suhr et al. (2019) also pointed out that "*Technomyrmex albipes*" is a frequently misidentified taxon that is often confused with multiple morphologically similar species. Additionally, *Technomyrmex* species vary substantially in feeding behaviour and pest status (Yamauchi et al. 1991; Warner 2003; Sunamura et al. 2022; Chen et al. 2024; Hsu et al. 2024), significantly impacting the choice of management strategies.

Hsu et al. (2024) reported a recent outbreak of *Technomyrmex albipes* alates in southern Taiwan where thousands of alates emerged during the summer, highly disturbing local residents. While the phenology of their nuptial flight swarms has been studied, several fundamental questions regarding genetics of the alate outbreak remain unaddressed. In this study, we first delineated *Technomyrmex* species collected in Taiwan (including ports-of-entry intercepts) using newly-generated *cytochrome c oxidase I* (COI) sequences, as well as those from the National Center for Biotechnology Information (NCBI) and the Barcode of Life Data Systems (BOLD). We then used single-nucleotide polymorphisms (SNPs) derived from ultra-conserved element (UCE) data to examine the genetic structure of *T. albipes* in Taiwan. To provide support to the evidence of hybridisation identified in our population genomic analysis, we conducted simulations to test whether the observed admixture patterns could be explained by hybridisation. We then discussed the potential role of hybridisation in the recent *T. albipes* outbreak.

Material and methods

Species identification using *Cytochrome C Oxidase I* (COI) barcodes

Five *Technomyrmex* species are currently known from Taiwan (Yamane et al. 2018). The *horni* group includes two species, *T. formosensis* and *T. horni*, which are typically found in low- to mid-disturbance areas. The *albipes* group comprises three species, amongst which *T. difficilis* is the least common, recorded only from southern Taiwan and from a small outlying island. Of the remaining two, *T. brunneus* has been documented across the Island, whereas *T. albipes* has, so far, only been recorded from the southern half of Taiwan. Based on our observations, *T. albipes* prefers forest edges rather than urban environments, similar to what has been reported for *T. brunneus* (Sunamura et al. 2024). Given this distribution pattern and our field observations, main sampling was conducted along a north–south transect following Provincial Highway No. 3, covering a broad range of forest-edge habitats. In total, 41 field-collected samples and 12 intercepted samples from Taiwanese ports-of-entry between 2017 and 2021 were included for the sequencing (Suppl. material 1). The global *Technomyrmex* key by Bolton (2007) was used for species identification, while the regional key by Yamane et al. (2018) was incorporated to distinguish the

recently-described *T. formosensis*, without conflict between the two. All samples were transferred to and preserved in absolute ethanol before DNA extraction.

One or two legs from the right side of the ant body were removed for DNA extraction. Gentra Puregene Tissue Kit (Qiagen, Venlo, the Netherlands) was employed following the manufacturer's protocol. Polymerase chain reaction (PCR) was conducted using Taq DNA Polymerase Master Mix RED (Ampliqon, Odense, Denmark). The standard barcode region of 658-bp *cytochrome c oxidase I* (COI) was amplified and sequenced following the primers LCO1490 and HCO2198 (Folmer et al. 1994). For samples subjected to ultra-conserved element sequencing (UCE, detailed below), COI sequences were retrieved from the contigs assembled by MEGAHIT v.1.2.9 (Li et al. 2015) and identified using MitoFinder v.1.4.2 (Allio et al. 2020) with the *min-contig-size* set to 200 and *blast-size* set to 20. For the reference used in MitoFinder, a mitochondrial genome of *T. albipes* was assembled using GetOrganelle v.1.7.7.0 (Jin et al. 2020) from whole-genome Illumina sequencing data of a single winged male (PW Hsu and CCS Yang, unpublished data) and annotated using MITOS2 (Bernt et al. 2013). In addition, 175 sequences were retrieved from the National Center for Biotechnology Information (NCBI) and 1,779 sequences were retrieved from the Barcode of Life Data System (BOLD) and incorporated into the analysis.

All sequences were aligned using the MAFFT online service (Katoh et al. 2019) and manually examined using a customised R script and MEGA7 (Kumar et al. 2016). Low-quality sequences that caused frameshifts due to indels or deletions or that contained high levels of missing data, were excluded from downstream analyses. If an individual was subjected to both Sanger and UCE sequencing, both sequences were included in the phylogenetic reconstruction to validate the results generated by MitoFinder; however, only the Sanger sequences were used in the haplotype network analysis. A Maximum Likelihood tree (ML) was inferred using RAxML v.8.2.12 (Stamatakis 2014). Data were partitioned, with each of the three codon positions assigned a separate GTRGAMMA substitution model and node support was calculated by 100 bootstrap replicates. Results of phylogenetic analyses were visualised using iTOL v.6 (Letunic and Bork 2021). For a total of 399 *T. albipes* sequences identified in the phylogeny that with no more than 40-bp missing sites, a minimum spanning haplotype network was built using PopART v.1.7 (Leigh and Bryant 2015).

Population genetic analysis using Ultra-conserved Elements (UCEs)

To further investigate the genetic structure of *T. albipes*, 29 ethanol-preserved and 15 dry-mounted samples were used for ultra-conserved element (UCE) sequencing (Suppl. material 1). All samples were morphologically identified to be *T. albipes* and some were additionally confirmed by COI barcoding. The protocol for DNA extraction and library preparation followed the protocol described in Hanisch et al. (2022) and Schultz et al. (2024). In short, destructive DNA extraction was conducted using DNeasy Blood and Tissue Kit (Qiagen, Venlo, the Netherlands) with overnight cell lysis to increase DNA yield. In the final step, DNA elution from the spin-columns was performed twice, each with 65 µl of nuclease-free water and subsequently quantified with a Qubit 4 Flex Fluorometer using 1× dsDNA High Sensitivity Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

For library preparation, up to 50 ng of DNA from each sample was used for sonic shearing with target fragment lengths being 300–600 bp using the Qsonica Q800

sonicator. Libraries were prepared using the Kapa Hyper Prep Library Kit (Kapa Biosystems, Wilmington, Massachusetts, USA), in which dual-indexing TruSeq adapters were used for ligation (Faircloth and Glenn 2012; Glenn et al. 2019). Clean-up steps were done by KAPA Pure Beads following Fisher et al. (2011). The library was amplified using KAPA HiFi ReadyMix with Illumina TruSeq primers. Each library was purified with Kapa Pure beads and quantified using the 1× dsDNA High Sensitivity Kit with a Qubit 4 Flex Fluorometer. Libraries were pooled and enriched by using the myBaits UCE Hymenoptera 2.5Kv2A kit, which targets 2,524 conserved loci (Branstetter et al. 2017), following the protocol provided in the Arbor Biosciences v.4.0.1 (https://arborbiosci.com/mybaits-manual/) and Borowiec (2019). Enriched libraries were quantified, pooled and sequenced using Illumina Hiseq 2500.

Raw reads were trimmed using Trimmomatic v.0.32 (Bolger et al. 2014) and examined using FastQC (Andrews 2010). Clean reads were mapped to a *T. albipes* reference genome (Size = 315.4 Mb, N50 = 19.5 Mb, BUSCO completeness of hymenoptera_odb10 = 97.7%; PW Hsu and CCS Yang, unpublished data) using Bowtie2 (Langmead and Salzberg 2012) and re-formatted using Samtools (Li et al. 2009). Variants were called using Bcftools v.1.16 (Li 2011) and subsequently filtered with Bcftools and a custom R script (Danecek et al. 2021). In Bcftools, variant filtering was carried out sequentially by first retaining only biallelic SNPs (-m2 -M2 -v snps). Subsequently, variants were filtered to include only those with a minor allele count of at least two (MAC ≥ 2). Sites were further selected if at least one individual had a sequencing depth between 20 and 1000 (FORMAT/DP ≥ 20 and FORMAT/DP < 1000). Finally, sites with more than 25% missing data were excluded (F_MISS < 0.25). In a subsequent step with the R script, individual SNPs with a depth of less than five were masked as missing. Then, the site-level filtering criteria were re-applied, excluding sites with more than 25% missing data and only those with a minor allele count not less than two were retained. Finally, individuals with more than one-third missing data were removed from the analysis. Population structure was inferred using sparse non-negative matrix factorisation (sNMF) with 100 iterations for each K value and alpha set at 500 using R package *LEA3* (Gain and François 2021). Euclidean distances between individual sample, as well as Nei's genetic distances (Takezaki and Nei 1996) between sNMF-inferred clusters were calculated using the R package *dartR* (Gruber et al. 2018) and visualised using the Neighbour-Joining method implemented in the R package *ape* (Paradis and Schliep 2019). Heterozygosity rates for individuals and sNMF-inferred clusters were calculated using the R package *dartR* (Gruber et al. 2018).

Characterising putative hybridisation between lineages using simulated data

To characterise putative admixed individuals, artificial diploids were simulated using a customised R script following the framework described by Cordonnier et al. (2019). The assumed hybridisation scenario, illustrated in Fig. 1, incorporates the life history of *T. albipes* group members. These ants exhibit two reproductive stages: one involving typical winged reproductives and another featuring wingless reproductives that mate exclusively within the nest (Yamauchi et al. 1991). Three samples were selected for each parental population, based on the ancestry coefficient estimated from sNMF, while individuals showing admixed patterns were classified as putative hybrids.

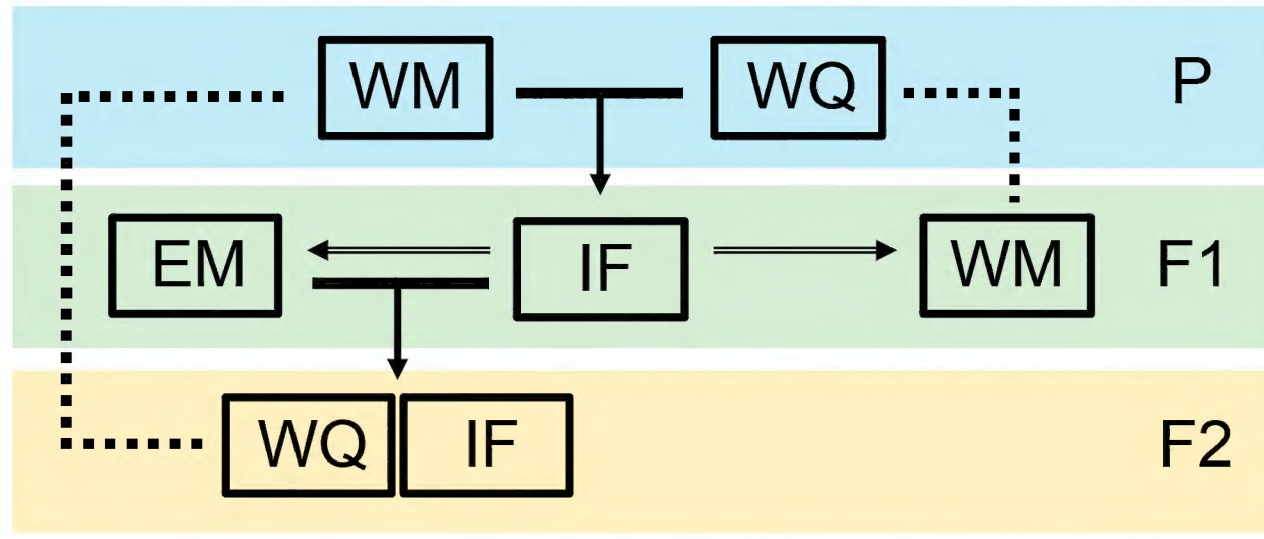

Figure 1. A hypothetical hybridisation scenario, based on the reproductive system of *Technomyrmex albipes*. A colony is initiated by a winged queen (WQ) mated with winged males (WM). Intercaste females (IF) are produced sexually (solid arrow) as first-generation offspring (F1) and subsequently take over reproductive roles within the colony. New males, including winged males and ergatoid males (EM), are produced through arrhenotoky (double-lined arrow) and are, therefore, considered as the same generation. New females, including WQ and IF, are produced through sexual reproduction between IF and EM and are classified as second-generation offspring (F2). Backcrossing (dashed line) may occur between F1 WM or F2 WQ and their respective parental counterparts (P).

For each locus, allele frequencies from the two parental populations were calculated and used to simulate hybrid individuals. Simulated datasets included pseudo-parental individuals (P0 and P1), first-generation hybrids (F1), second-generation hybrids (F2) and backcrossed hybrids between F1 or F2 and one of the parental populations (Fig. 1). For each category, ten individuals were simulated. P0, P1 and F1 individuals were generated by sampling alleles, based on the calculated frequencies of the parental populations. F2 individuals were assumed to originate from inbreeding between intercaste females and ergatoid males. Therefore, genotypes of each F2 individual were calculated from one F1 individual, with alleles assigned according to Mendelian inheritance: homozygous loci remained unchanged, while heterozygous loci had a 50% probability of remaining heterozygous and a 25% probability of becoming homozygous for either allele. Backcrossing was simulated between F1 winged males or F2 winged queens and the parental populations (Fig. 1). Allele frequencies from the F1 and F2 populations were calculated to simulate backcrossed hybrids. To mimic field data, 10% of the genotypes in each simulated individual were randomly masked as missing after all simulations.

Ancestry coefficients of parental and simulated individuals were estimated using sNMF under the same settings. Additionally, NewHybrids (Anderson and Thompson 2002) was used to infer hybrid categories, implemented through the R package *dartR*. The analysis was conducted using 500 randomly selected loci, with a burn-in of 50,000 iterations and 100,000 sweeps. Parental populations were left unassigned in the inference.

Results

Species identity of worldwide *Technomyrmex* and genetic variation of global *Technomyrmex albipes*

A total of 97 COI sequences were newly generated in this study (GenBank accession numbers PV483596–PV483693, Suppl. material 1), comprising 59 individuals collected across the Taiwan Island, 12 individuals from Taiwanese ports

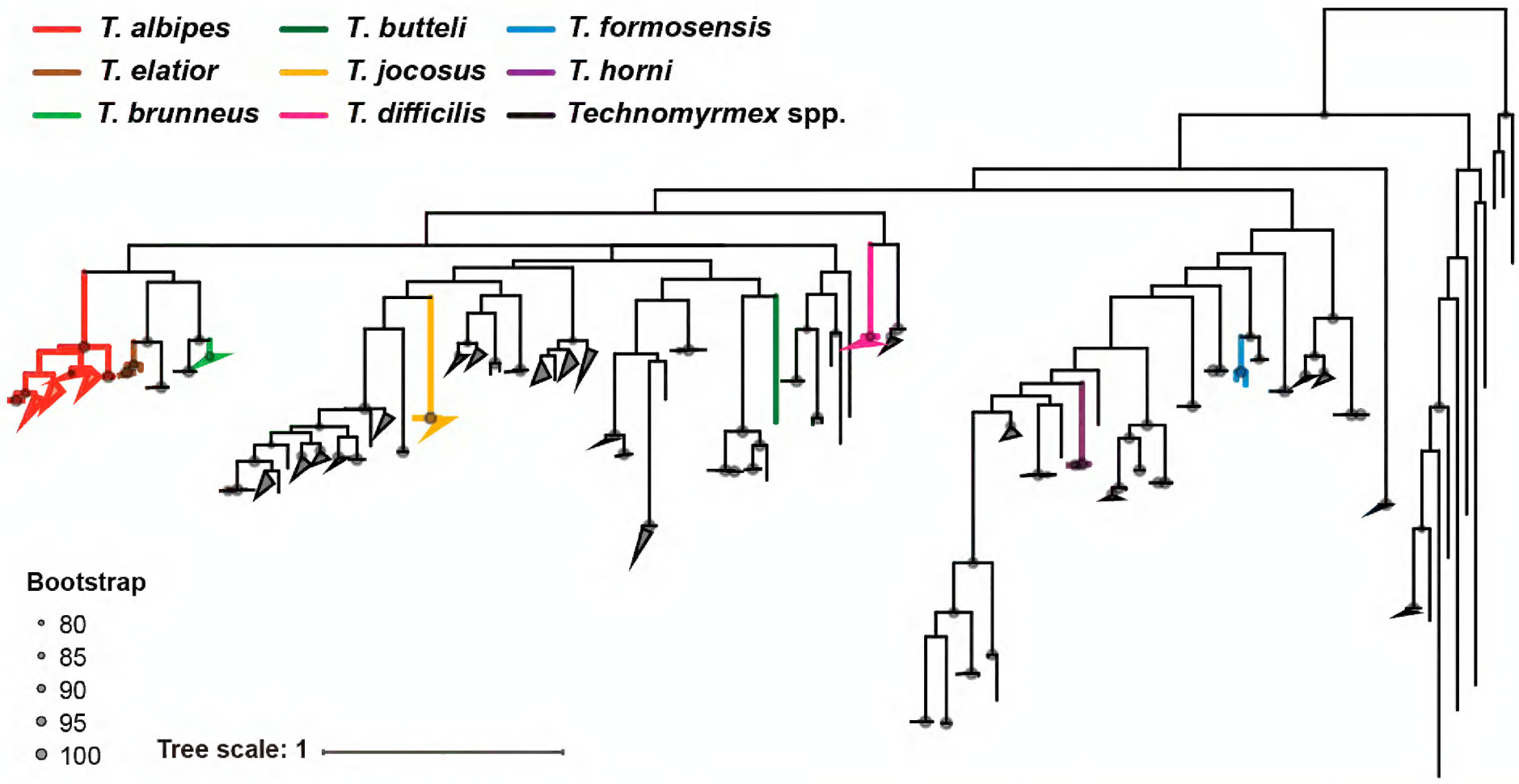

Figure 2. Phylogenetic analyses of worldwide *Technomyrmex*, based on *cytochrome c oxidase I* (COI) barcode sequences. A Maximum Likelihood (ML) tree was constructed from 1,796 samples using RAxML, with black circles marking nodes supported by bootstrap values > 80. The eight species collected in Taiwan or intercepted at Taiwanese ports of entry between 2017 and 2021 are highlighted with coloured branches. Branches with length < 0.1 are collapsed to improve visualisation.

of entry, and 15 individuals from foreign localities, obtained using either Sanger (n = 53) or UCE sequencing (n = 44). One *Tapinoma* sample was also included as an outgroup. These sequences were combined with public data to infer a Maximum Likelihood (ML) phylogeny based on 1,796 COI sequences of 658 bp in length (Fig. 2; details in Suppl. material 2). Despite weak support for deeper nodes, strong support for more recent nodes allowed for robust species delimitation of Taiwanese *Technomyrmex* using COI barcodes. Newly-generated sequences from Taiwanese field-collected samples were divided into five groups and some of which clustered with sequences of exotic samples retrieved from the databases. The five groups correspond to each of the five species including *albipes*, *brunneus* and *difficilis* from the *albipes* species group and *horni* and *formosensis* from the *bicolor* species group. The clustering pattern was consistent with that based on morphology described by Bolton (2007) and Yamane et al. (2018). While most Taiwanese ports-of-entry samples were clustered with their own species counterparts found in Taiwan, a subset of samples belonged to three additional clades that have not been reported in Taiwan (Table 1). Of these, four specimens, intercepted from Thailand, formed a distinct group that was not grouped with any other known sequences in this study and they were identified morphologically as *T. elatior*. One sample intercepted from plants originating from Indonesia was grouped near *T. anterops* from the database and morphologically identified as *T. butteli.* One dealate queen intercepted from New Zealand clustered with other samples from Zealand and Australia that were labelled as *T. jocosus*. Due to the inability to identify the sample based on the current knowledge of *Technomyrmex* queens, it was identified here as *T. jocosus*, based on molecular data and its geographic distribution.

Our phylogenetic analysis identified 437 sequences as *T. albipes*, of which 399 with less than 40 missing sites were retained for haplotype network reconstruction (Fig. 3).

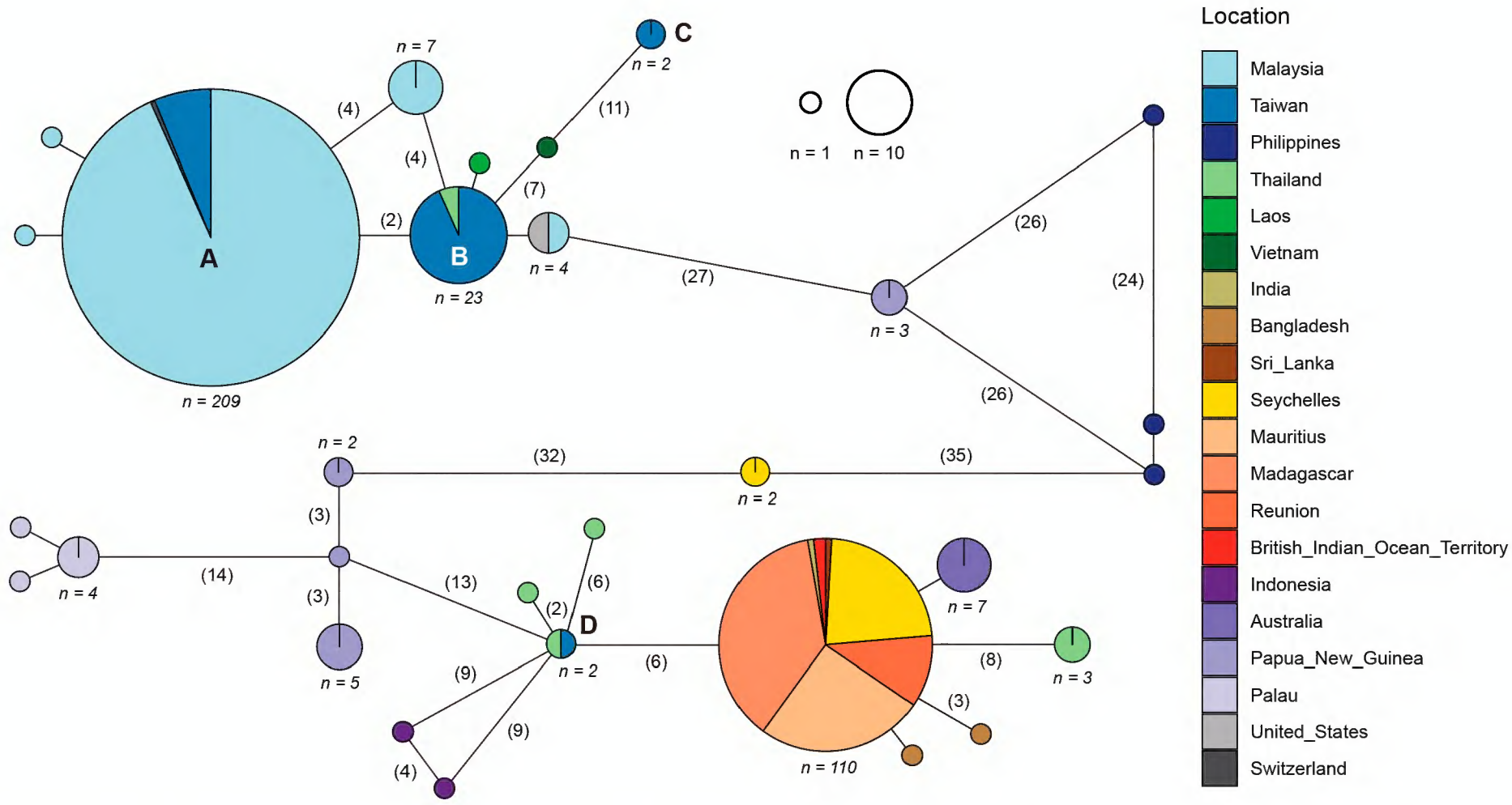

Figure 3. Haplotype network of *Technomyrmex albipes* based on *cytochrome c oxidase I* (COI) barcode sequences from 399 (with < 40 missing sites) out of 437 samples identified in the phylogenetic tree. The network was constructed in POPART using the minimum spanning method with a total of 618 unmasked sites. Haplotypes detected in Taiwan are labelled as A, B, C and D. The number of mutational steps between haplotypes is indicated in parentheses, while unlabelled connections represent a single mutational step. Sample metadata and geographic origins are provided in Suppl. material 3.

Table 1. Documented occurrences of the eight *Technomyrmex* species analysed in this study. The symbol "O" indicates verified records for each species in Taiwan and at Taiwanese ports of entry.

Species	Recorded in Taiwan by Yamane et al. (2018)	Recorded in Taiwanese port-of-entry during 2017–2021
T. albipes	O	O
T. brunneus	O	O
T. difficilis	O	O
T. formosensis	O	
T. horni	O	
T. butteli		O
T. elatior		O
T. jocosus		O

These sequences comprised 30 haplotypes, with two major haplotypes dominating the dataset: one associated with samples from Malaysia, Taiwan and Switzerland (n = 209) and the other with samples from the Indian Ocean Region (n = 110). The two major haplotypes were separated by a large number of mutational steps, with several samples from Australasia and Indomalaya occupying intermediate positions. Four haplotypes distributed in Taiwan were named A to D, with A and B being the most dominant. Haplotypes identified from other regions were designated H1 to H26, based on descending sample size (Suppl. material 3).

Population structure of *Technomyrmex albipes* in Taiwan and admixture of different lineages

Illumina sequencing of 44 *T. albipes* samples generated between 623,217 to 5,529,443 paired raw reads (median = 3,216,176). All raw reads have been deposited in the Sequence Read Archive (SRA) under NCBI BioProject PRJNA1251141. Single nucleotide polymorphisms (SNPs) were identified and filtered, resulting in a final dataset of 39 individuals and 4,086 variable sites. In addition to nuclear data, we recovered COI sequences from each UCE dataset using MitoFinder. For the 1,515 bp COI sequence, we retrieved either full or partial sequences from all samples (ranging from 333 to 1,515 bp, with a mean length of 1,325 bp). Notably, samples sequenced by both Sanger and UCE showed identical sequences across the 658-bp barcode region. These mitochondrial sequences were subsequently used in the phylogenetic and haplotype network analyses described above.

To understand population structure, sNMF analysis was conducted to infer the optimal number of hypothetical ancestral populations (K) with SNP data. For each K, a hundred runs were analysed and the one with the minimum cross-entropy was presented (Figs 4, 5). The lowest cross-entropy value was observed at K = 2, separating Southeast Asian samples from the others. At K = 6, although some non-Taiwanese samples could not be confidently assigned to clusters due to limited sample sizes (e.g. UCE_05), Taiwanese samples could mostly be assigned into four clusters, hereafter named as TW-North (green), TW-Central (peach), TW-South (orange) and TW-East (purple), based on the sNMF clustering and their geographical distribution. Fig. 6 summarises the sNMF clustering, haplotypes and their geographic distributions in Taiwan. Individuals from TW-North and TW-South harbour haplotype A and are geographically separated by individuals from TW-Central, which harbour haplotype B. Interestingly, several individuals exhibited admixed genotypes, showing ancestry coefficients from both TW-North/TW-South and TW-Central clusters (red-highlighted in Figs 5, 6). These admixed

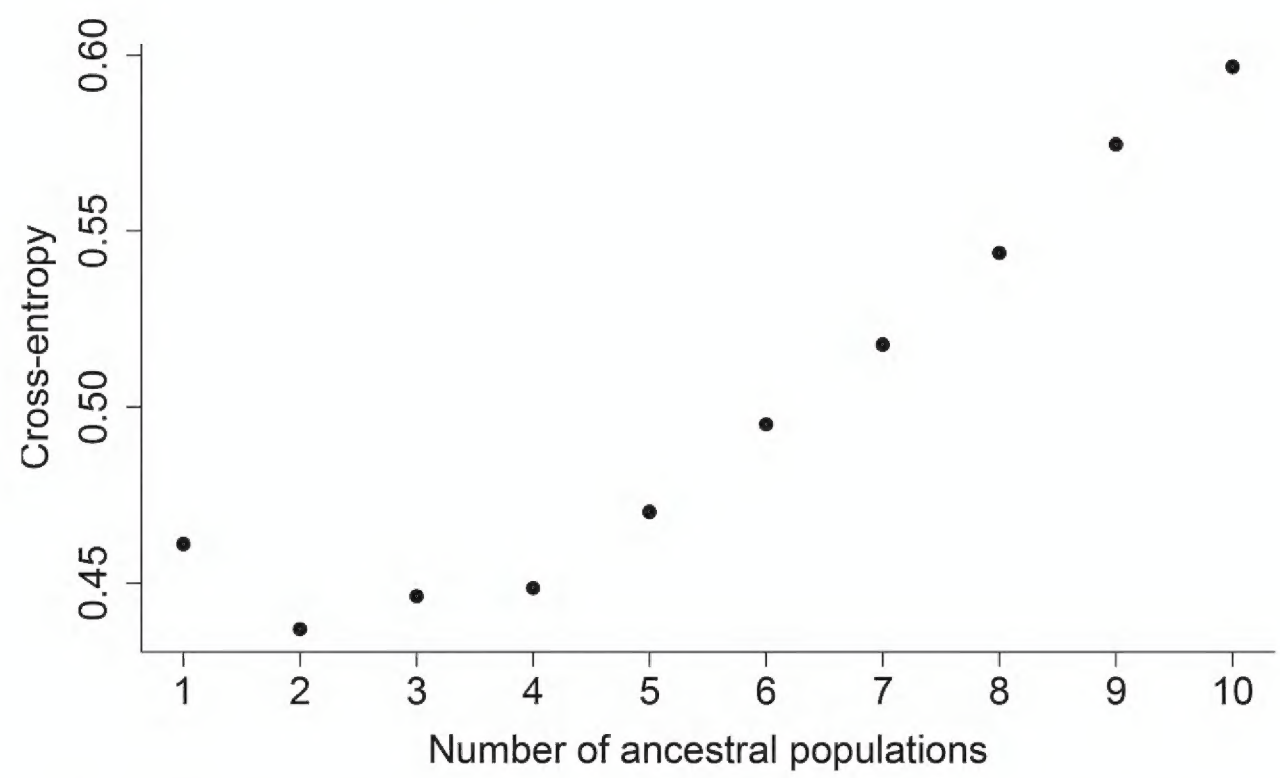

Figure 4. Minimum cross-entropy in each number of hypothetical ancestral populations. The admixture analysis is performed with sparse non-negative matrix factorisation (sNMF) with a hundred runs for each K.

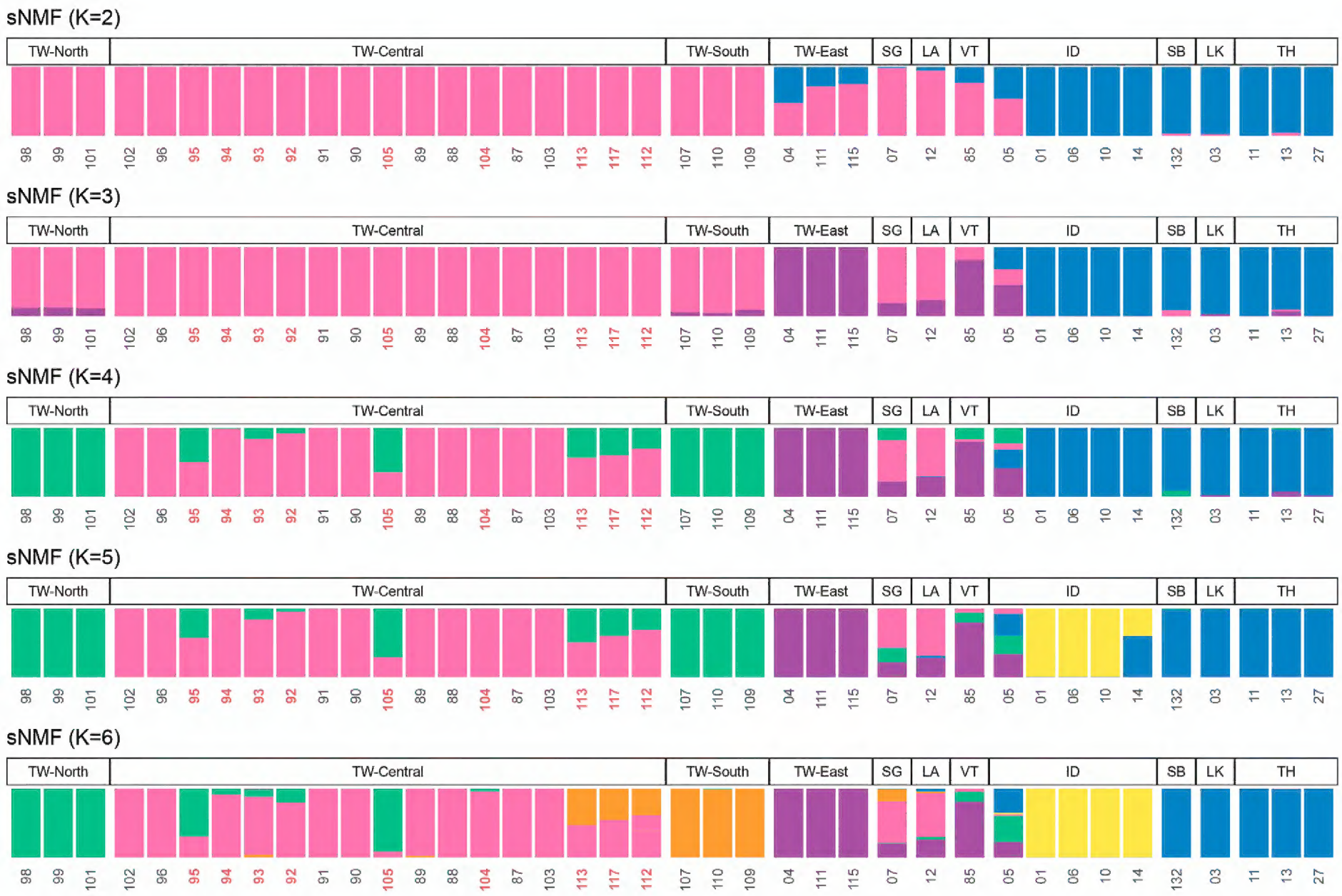

Figure 5. Ancestral coefficients of *Technomyrmex albipes* samples computed with sparse non-negative matrix factorisation (sNMF). In a total of 4,086 variable sites from 39 samples are used for analysis, with a maximum missing rate of 25% per site and one-third per individual. In a hundred runs of sNMF for each number of hypothetical ancestral populations (K), the run with minimum cross-entropy is shown. Samples are grouped by geographic region. Sample codes are shown at the bottom of each coefficient bar. Individuals from TW-Central with mixed ancestry coefficients > 0.03 at K = 6 are highlighted in red and classified as TW-PutativeHybrid. Abbreviations: TW = Taiwan, SG = Singapore, LA = Laos, VT = Vietnam, ID = Indonesia, SB = Solomon Islands, LK = Sri Lanka, TH = Thailand.

individuals, with any non-TW-Central ancestry coefficient exceeding 0.03, were classified as TW-PutativeHybrid. TW-East samples form a genetically distinct cluster, characterised by haplotypes C and D.

Individual-level and cluster-level genetic distances are summarised in Fig. 7, while heterozygosity is shown in Fig. 8 and Suppl. material 4. TW-North and TW-South are more closely related to each other (Fig. 7A) and also exhibit similar levels of heterozygosity, both of which are lower than those of TW-Central (Fig. 8). TW-PutativeHybrid, although genetically admixed, displays a heterozygosity level comparable to that of TW-Central. Individuals with distinct admixture patterns did not show elevated levels of heterozygosity. While TW-PutativeHybrid as a whole is genetically closer to TW-Central, several individuals (e.g. UCE_105, UCE_112, UCE_113 and UCE_117) appear genetically closer to TW-North or TW-South (Fig. 7B). Notably, UCE_112 harbours haplotype A, which is otherwise found only in TW-North and TW-South populations.

To examine the status of TW-PutativeHybrid individuals, we simulated diploid individuals for each of the following categories: P0, P1, F1, F2 and F1 × parent (or F2 × parent) backcrosses with two parental populations (Fig. 1). Each parental population was represented by three individuals. Inferences were performed using both sNMF and NewHybrids under three crossing scenarios: (1) TW-North ×

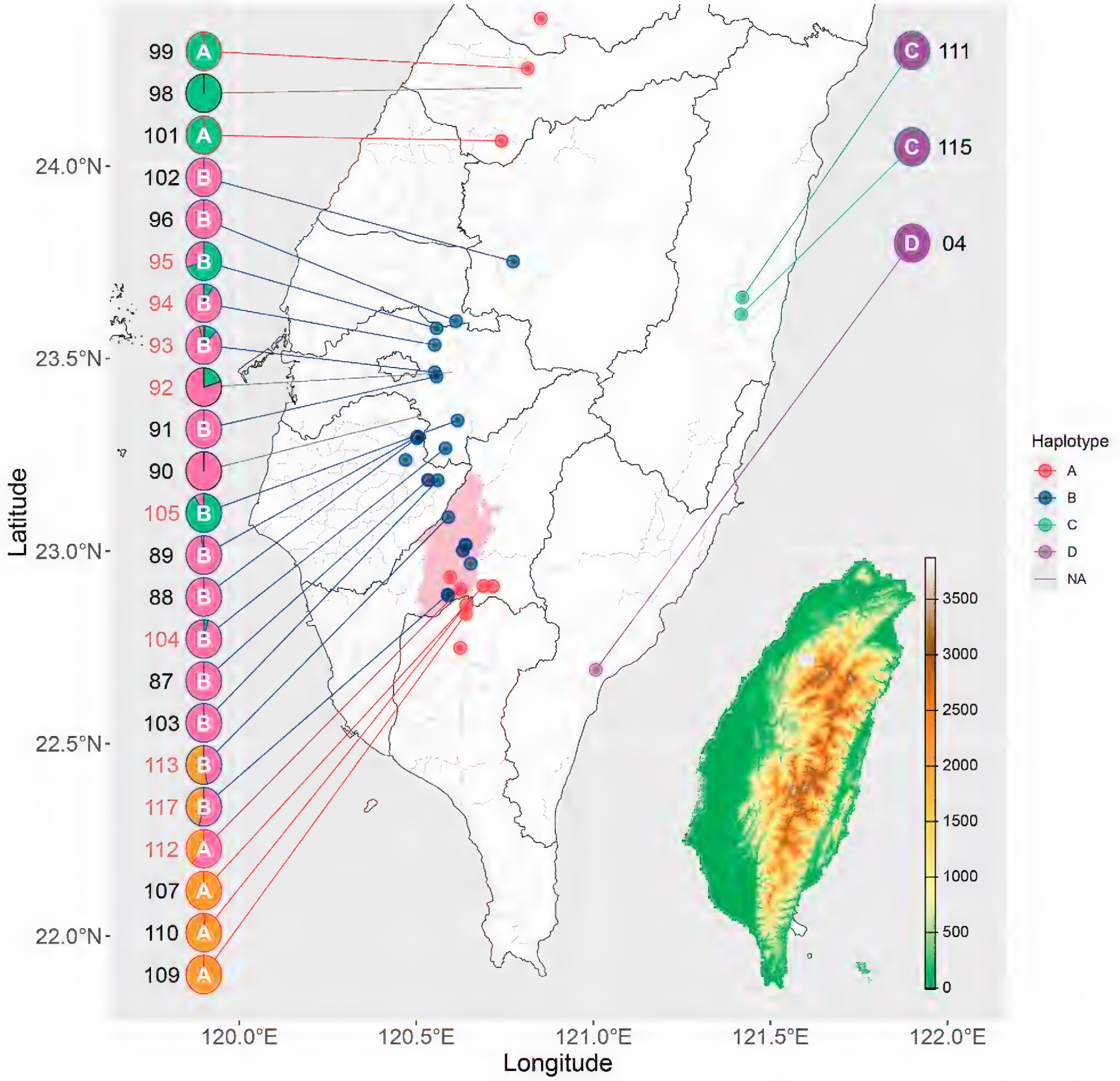

Figure 6. Population structure of *Technomyrmex albipes* in Taiwan. Ancestry coefficients from K = 6 (Fig. 5) are represented as pie charts, with UCE sample codes shown adjacent to each chart. *Cytochrome c oxidase I* (COI) haplotypes A, B, C and D (Fig. 3) are labelled directly on the charts and also indicated by the colour of connecting dots and lines. Individuals classified as TW-PutativeHybrid (Fig. 5) are highlighted with red sample codes. Four administrative districts marked in pink correspond to study sites from Hsu et al. (2024), where massive nuptial flight swarms were observed.

TW-Central, (2) TW-South × TW-Central and (3) TW-East × Thailand as reference. Our analyses showed that using either F1 × parent or F2 × parent backcrosses yielded comparable results. Therefore, we present the former in the main text (Fig. 9) and the latter in Suppl. material 5.

Across all simulations (P0, P1, F1, F2 and two backcross types), sNMF and NewHybrids produced consistent patterns in all three cross scenarios. In the simulated data, sNMF clearly assigned simulated P0 and P1 individuals to their respective categories, assigned F1 and F2 individuals with nearly equal ancestry coefficients from the two parental populations and assigned backcross individuals with ancestry coefficients biased towards the parental population with which they were crossed.

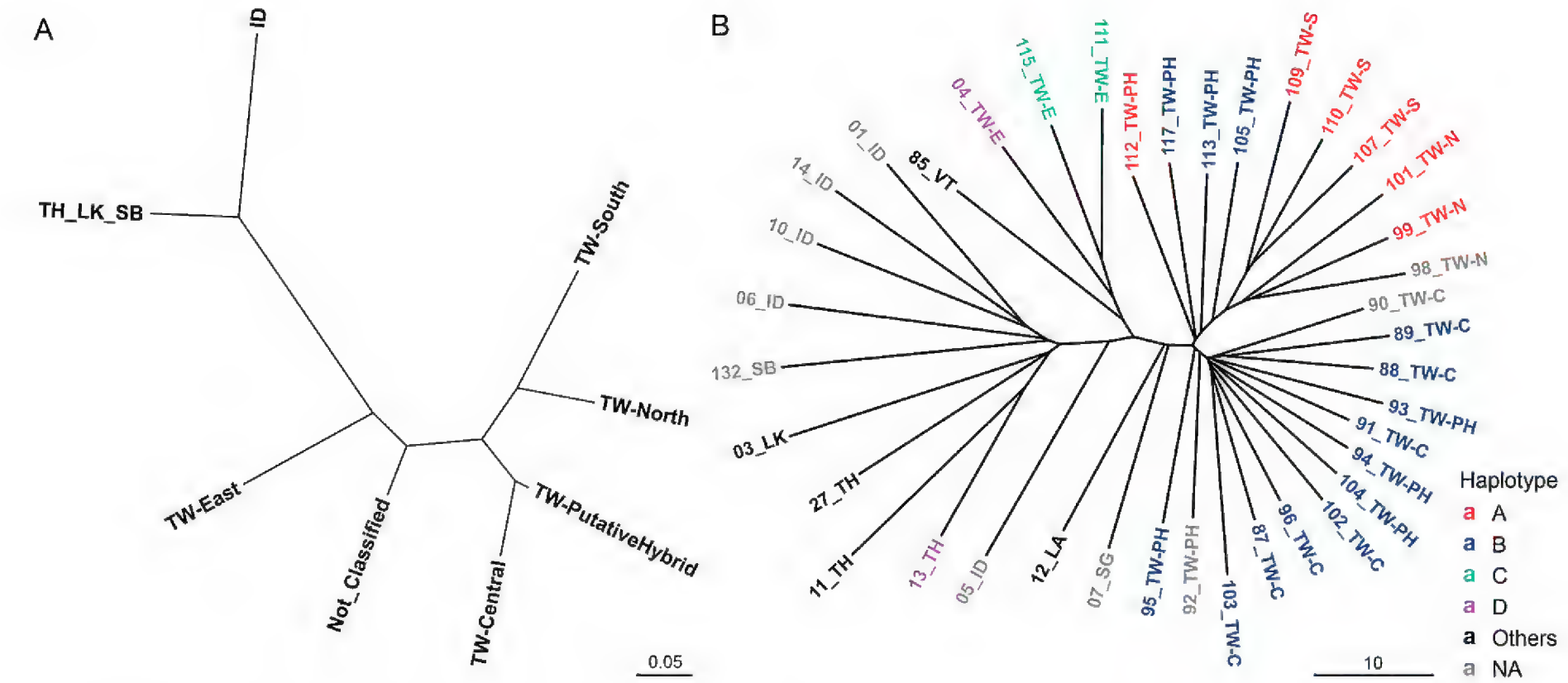

Figure 7. Neighbour-Joining trees, based on genetic distances amongst *Technomyrmex albipes* samples. **A.** Nei's genetic distances amongst sNMF-inferred clusters from Fig. 5; **B.** Euclidean genetic distances amongst individual samples. Tips are sample codes followed by their assigned sNMF clusters. Colours represent their *cytochrome c oxidase I* (COI) haplotype: A (red), B (blue), C (green), D (purple), Others (black) and NA (grey). Abbreviations: TW-N = TW-North, TW-C = TW-Central, TW-S = TW-South, TW-E = TW-East, TW-PH = TW-PutativeHybrid, LA = Laos, LK = Sri Lanka, ID = Indonesia, SB = Solomon Islands, TH = Thailand, VT = Vietnam.

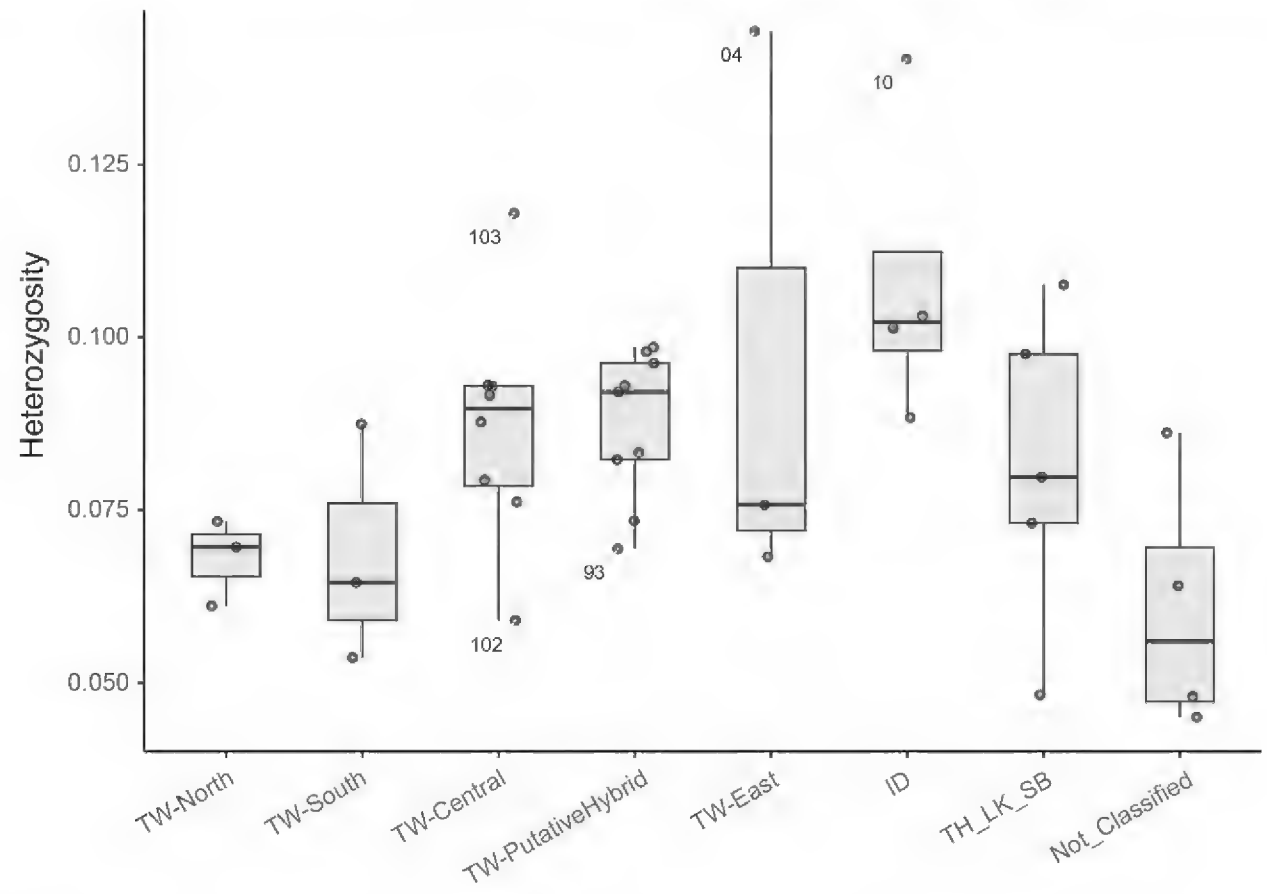

Figure 8. Observed heterozygosity (Ho) of *Technomyrmex albipes* samples. Individual heterozygosity rates are shown as dots and grouped by sNMF-inferred clusters from Fig. 5. Some extreme values are annotated with sample codes. The original data can be found in Suppl. material 4.

NewHybrids also provided clear categorisation of simulated individuals into their expected hybrid classes, with only a few backcross individuals assigned as F2.

In our empirical data, sNMF clustering showed slight differences from the initial pattern presented in Fig. 4, with both TW-Central and TW-PutativeHybrid individuals showing higher ancestry coefficients from TW-North/TW-South. However, TW-PutativeHybrid individuals did show higher ancestry coefficients from TW-North/TW-South than those of both TW-Central, with a few individuals consistently showing nearly equal ancestry coefficients. For NewHybrids, while

Figure 9. Classification of field and simulated *Technomyrmex albipes* individuals under a hypothetical hybridisation scenario described in Fig. 1. Simulations were conducted for three pairwise combinations of sNMF-inferred clusters: TW-North × TW-Central (top panel), TW-South × TW-Central (middle panel) and a reference cross of TW-East × TH (bottom panel). Artificial diploid individuals were generated based on allele frequencies from two parental populations (P0 and P1 shown as the first two categories on the left). Simulated categories include pseudo-parental individuals (sim_P0 and sim_P1), first-generation hybrids (sim_F1), second-generation hybrids (sim_F2) and backcrosses between F1 and parental populations (sim_F1BC), with ten individuals per category. Classification results are shown, based on ancestry coefficients estimated using sNMF (K = 2, upper row) and posterior probabilities for six hybrid categories inferred by NewHybrids (lower row).

several individuals from TW-Central and TW-PutativeHybrid were assigned to backcross categories, multiple individuals were still assigned as F2 hybrids. Considering both analyses, individuals UCE_113 and UCE_117 showed consistent admixture signals between the two parental populations.

Discussion

Ongoing global invasion of *Technomyrmex* species highlights needs for molecular tools in pest management

Technomyrmex species, particularly those in the *albipes* species group, possess significant invasive potential, but have been overlooked until recently (Putri and Cronin 2023; Hsu et al. 2024). They have been frequently recorded at ports of entry

and in greenhouses (e.g. Wetterer (2013); Suhr et al. (2019); Freyhof and Janke (2024)), reflecting both high establishment potential and propagule pressure. In our dataset of 529 interception records from Taiwan between 2017 and 2021, 19 were identified as *Technomyrmex* (3.6%), ranking amongst the top 10 most intercepted genera. Similar trends have also been observed in other ports-of-entry interceptions from major continental regions, including North America, Europe and Australia (Suarez et al. 2005; Ward et al. 2006; Boer and Vierbergen 2008; Suhr et al. 2019), suggesting ongoing global threats from *Technomyrmex* invasion. In this study, we established a COI barcoding dataset for worldwide tramp *Technomyrmex* species and successfully linked seven morphologically identified species to corresponding genetic clusters (Fig. 2 and Suppl. material 2). This dataset provides a molecular foundation for a global *Technomyrmex* species identification toolkit.

Technomyrmex albipes is arguably the most frequently misused name in previous literature and online databases to describe a set of *Technomyrmex* species that are morphologically similar (Yamauchi et al. 1991; Warner 2003; Bolton 2007; Wetterer 2013; Suhr et al. 2019). Our COI dataset, coupled with recent taxonomic revisions of *Technomyrmex* in Taiwan and globally (Bolton 2007; Yamane et al. 2018), confirms with confidence that the species responsible for the massive nuptial flight swarming reported in southern Taiwan is indeed *T. albipes*. We also found that it is an extremely widespread species, representing nearly one-quarter of all *Technomyrmex* sequences in the databases spanning across the Malagasy, Indomalayan and Australasian Regions (Fig. 3). Notably, two interception records from Taiwanese Custom (Suppl. material 1) and two records from established populations in the United States (Hawaii) and Switzerland (Fig. 3) provide direct evidence of both the introduction and establishment phases of *T. albipes*, highlighting its threats across multiple regions.

In addition to *T. albipes*, *T. brunneus* and *T. difficilis* that have already been recorded as pests (Warner 2003; Putri et al. 2021; Terayama et al. 2021; Hsu et al. 2024), we also identified three additional species with invasive potential, namely *T. elatior*, *T. butteli* and *T. jocosus*, all of which are in our ports-of-entry dataset and already reported to have intercaste females in Bolton (2007) (Table 1 and Fig. 2). This raises concern about other members of the *albipes* group as potential emerging invaders. Furthermore, although *T. albipes* is the most commonly misused name, two other names, *T. vitiensis* and *T. pallipes*, warrant further attention as they are scattered across multiple branches in our phylogeny. Due to the difficulty of identifying these species solely based on morphology, further genetic evidence is necessary to complement the current barcoding toolkit for tramp *Technomyrmex* species.

Human-mediated dispersal of *Technomyrmex albipes* in- and outside its native range

According to AntMaps (Janicki et al. 2016), *T. albipes* is presumed to be native to the Indomalayan Region, in which Taiwan is situated at the north-eastern edge of this bioregion. Our analysis of genetic variation in Taiwanese *T. albipes* populations supports this hypothesis. The Central Mountain Range, which runs north-south through Taiwan (Fig. 6), serves as a major geographic barrier limiting the dispersal of indigenous species between east and west. This has led to pronounced differences in fauna and flora between the two regions, as well as frequent genetic divergence within species (Wu et al. 2010; Chang et al. 2015; Hwang et al. 2021). The genetic structure of Taiwanese *T. albipes* showed a similar pattern, with the

eastern population (TW-East) being genetically distinct from those in the west. This suggests restricted gene flow caused by the Central Mountain Range and that the eastern population may have undergone a separate dispersal or evolutionary history. This is further supported by the low levels of human disturbance in eastern Taiwan, which makes it more likely that TW-East represents a naturally-occurring population, rather than an introduced one. Finally, the broad and continuous distribution of *T. albipes* in areas surrounding Taiwan, together with the spatial patterning of haplotypes (Fig. 3), reinforces the hypothesis of its native status.

Interestingly, we also found an "truncated" distribution of *T. albipes* genetic structure across western Taiwan: TW-North and TW-South, two genetically similar populations as revealed by both COI and UCE data, were geographically separated by a genetically distinct population, TW-Central (Figs 6, 7). While multiple scenarios could explain this pattern, the simplest ones involve at least one human-mediated dispersal event. One possibility is that all three lineages are native to Taiwan and colonies from the north were transported by humans to southern Taiwan (or vice versa). Alternatively, the presence of COI haplotypes A and B in both western Taiwan and mainland Southeast Asia raises the possibility that one or more of these lineages may have been introduced from outside Taiwan, resulting in the observed disjunct distribution. Other hypotheses which do not involve human activity, such as natural dispersal with the current distribution representing post-glacial refugia or local adaptation driven by differences in social organisation, cannot be excluded, but require more data for support. Unfortunately, our UCE dataset covered only a small fraction of the global distribution of *T. albipes* and precluded us from drawing conclusions. The closest samples to those of TW-Central and TW-PutativeHybrid were UCE_07 from Singapore and UCE_12 from Laos (Fig. 7), with UCE_12 sharing a COI haplotype that differed from haplotype B by one SNP (Fig. 3) and both clustering near, but not within the western Taiwan samples. However, COI sequences from public databases showed that haplotypes A and B were identical to those from Malaysia and Thailand, respectively (Fig. 3). Therefore, more samples across mainland Southeast Asia are needed to pinpoint the putative origin of the Taiwanese *T. albipes* lineages and to confirm either hypothesis.

Admixture between lineages and ongoing outbreaks of *Technomyrmex albipes*

To further investigate the potential admixture observed in the initial sNMF analysis (TW-PutativeHybrid, red-highlighted in Figs 5, 6), we simulated diploid individuals under hypothetical hybridisation scenarios and compared them with real samples using both sNMF and NewHybrids (Fig. 9). Specifically, individuals UCE_92, UCE_95 and UCE_105 exhibited clear admixture between TW-North and TW-Central, while UCE_112, UCE_113 and UCE_117 showed admixture between TW-South and TW-Central. Under the corresponding cross scenario, these individuals generally exhibited ancestry coefficients and hybrid class assignments resembling those of simulated F2 hybrids, with their genetic composition evenly derived from the two parental populations. Interestingly, TW-South × TW-Central hybrids were collected within the contact zone that perfectly overlaps with the region where alate outbreaks of *T. albipes* were first reported in Hsu et al. (2024) (districts marked in pink in Fig. 6).

What causes the alate outbreaks of *T. albipes*? A straightforward explanation might involve abrupt population expansion driven by climate change or human

disturbance (Spear et al. 2021). Since caste determination in ants is primarily influenced by environmental factors (Wheeler 1986), changes in microhabitat may also increase alate production without direct effects on population size. However, given that genetic factors are also known to contribute to caste differentiation (Anderson et al. 2008; Schwander et al. 2010), an alternative and intriguing hypothesis is that admixture between different lineages may have facilitated the outbreaks. Recent studies suggested that introduction of a genetically distinct lineage can promote the proliferation of a resident population through hybridisation (Jeffery et al. 2017; Yamaguchi et al. 2019; Corrêa et al. 2019). While it remains unclear whether and how lineage admixture can trigger the *T. albipes* alate outbreak, logical subjects of further investigation include the potential role of genetic caste determination (Anderson et al. 2008; Schwander et al. 2010), heterosis (Lippman and Zamir 2007; Wagner et al. 2016), relief from deleterious genetic loads or pathogens (Vilà et al. 2000; Eastwood et al. 2014) or increased genetic diversity and accelerated evolution (Hodgins et al. 2018; Adavoudi and Pilot 2021).

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

In preparing this manuscript, generative AI tools, specifically ChatGPT (OpenAI) and Claude (Anthropic), were employed to assist with draft code generation for simulations, figure preparation, and grammar correction. All AI-generated content was carefully reviewed and edited by the authors to ensure accuracy and readability. No text, code, or figures generated by AI were used directly without human oversight and modification.

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Author contributions

PWH contributed to Conceptualization, Data Curation, Formal Analysis, and Methodology. JSC contributed to Data Curation, Resources, and Methodology. SPT contributed to Methodology and

Formal Analysis. SY, FCH, JKW, and TRS contributed to Resources. TRS and CCL contributed to Funding Acquisition. SD contributed to Funding Acquisition and Supervision. CCSY contributed to Conceptualization, Funding Acquisition, and Supervision. While PWH and CCSY contributed to the Original Draft Preparation, Review & Editing was carried out by all other authors.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Sample information for *Technomyrmex albipes* and related species used in this study

Authors: Po-Wei Hsu, Jeffrey Sosa-Calvo, Shu-Ping Tseng, Seiki Yamane, Feng-Chuan Hsu, James K. Wetterer, Ted R. Schultz, Chung-Chi Lin, Shigeto Dobata, Chin-Cheng Scotty Yang

Data type: xlsx

Explanation note: The table includes sample codes, Genbank accession numbers, haplotype assignments, and other collection metadata for all samples used for Sanger and ultra-conserved element (UCE) sequencing.

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Link: https://doi.org/10.3897/neobiota.101.162972.suppl1

Supplementary material 2

Complete phylogeny of *Technomyrmex* based on *cytochrome c oxidase I* (COI) barcode sequences, corresponding to the tree shown in Fig. 1

Authors: Po-Wei Hsu, Jeffrey Sosa-Calvo, Shu-Ping Tseng, Seiki Yamane, Feng-Chuan Hsu, James K. Wetterer, Ted R. Schultz, Chung-Chi Lin, Shigeto Dobata, Chin-Cheng Scotty Yang

Data type: pdf

Explanation note: The maximum likelihood (ML) tree was constructed from 1,796 samples using RAxML, with nodes annotated with bootstrap values. The eight species detected in Taiwan or intercepted at Taiwanese ports of entry between 2017 and 2021 are highlighted with colored branches.

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Link: https://doi.org/10.3897/neobiota.101.162972.suppl2

Supplementary material 3

Haplotype assignments corresponding to the *cytochrome c oxidase I* (COI) network shown in Fig. 3

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Data type: csv

Explanation note: The table includes sample codes, haplotype names A–D (for Taiwanese samples) and H1–H26 (for non-Taiwanese samples), along with their geographic distributions.

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Link: https://doi.org/10.3897/neobiota.101.162972.suppl3

Supplementary material 4

Summary of heterozygosity and inbreeding statistics for each *T. albipes* group, calculated at the cluster and individual levels using the *gl.report.heterozygosity()* function from the R package *dartR*

Authors: Po-Wei Hsu, Jeffrey Sosa-Calvo, Shu-Ping Tseng, Seiki Yamane, Feng-Chuan Hsu, James K. Wetterer, Ted R. Schultz, Chung-Chi Lin, Shigeto Dobata, Chin-Cheng Scotty Yang

Data type: csv

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Link: https://doi.org/10.3897/neobiota.101.162972.suppl4

Supplementary material 5

Classification of field and simulated *Technomyrmex albipes* individuals under a hypothetical hybridization scenario described in Fig. 1

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Data type: svg

Explanation note: Simulations were conducted for three pairwise combinations of sNMF-inferred clusters: TW-North × TW-Central (top panel), TW-South × TW-Central (middle panel), and a reference cross of TW-East × TH (bottom panel). Artificial diploid individuals were generated based on allele frequencies from two parental populations (P0 and P1 shown as the first two categories on the left). Simulated categories include pseudo-parental individuals (sim_P0 and sim_P1), first-generation hybrids (sim_F1), second-generation hybrids (sim_F2), and backcrosses between F2 and parental populations (sim_F2BC), with ten individuals per category. Classification results are shown based on ancestry coefficients estimated using sNMF (K = 2, upper row), and posterior probabilities for six hybrid categories inferred by NewHybrids (lower row).

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